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Rearing Doryphorophaga doryphorae a Tachinid Parasite of the Colorado Potato Beetle, Leptinotarsa decemlineata

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ABSTRACT

The tachinid, *Doryphorophaga doryphorae* (Riley), a parasite of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, was successfully reared for six generations in the laboratory. Access to sunlight stimulated mating of the parasite in cages. Fertility of the female parasite was determined by dissection of the larvae of the exposed beetle. Thirty-seven percent of the CPB larvae exposed to the fertile parasites developed into flies.

The most expedient rearing procedure involved the selection of only fertile female parasites. Therefore, a CPB larvae exposed to a presumed recently mated fly was dissected for parasite larvae.

KEYWORDS: *Doryphorophaga doryphorae*, Colorado potato beetle, *Leptinotarsa decemlineata*, biological control, potatoes.

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REARING DORYPHOROPHAGA DORYPHORAE, A TACHINID PARASITE OF THE COLORADO POTATO BEETLE, LEPTINOTARSA DECEMLINEATA [ ].

By George Tamaki, R. L. Chauvin, and Ting Hsiao[1]

INTRODUCTION

In the Pacific Northwest, the two major natural enemies of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say, are the pentatomid predator, *Perillus bioculatus* (Fabricius), which primarily attacks the egg and larval stages of the CPB, and a tachinid fly, *Doryphorophaga doryphorae* (Riley), a CPB larval parasite. In 1960, Kelleher completed a 3-year study on the life history and ecology of *D. doryphorae* and reported that he was not successful in his attempt to rear the fly to adulthood. Bruneteau (*1937*),[2] Feytaud (*1938*), and Trouvelot (*1932*) reported rearing *D. doryphorae*, but they were only able to rear the fly for one generation. More recently, Billotti and Persoons (*1965*) reported rearing the parasite, but the number of parasites obtained from each generation was low.

The CPB is considered a major pest in most potato producing countries in the world and, since CPB is insecticide resistant in Eastern and Western Europe and Eastern United States (Cutkomp et al. *1958* and Lakocy *1967*), control is difficult. It appears to be only a matter of time before CPB populations in the Pacific Northwest will become insect resistant with the potential to defoliate many fields of potatoes. Therefore, we are now emphasizing development of alternative control measures, such as expanding the natural enemy complex by importation of new species and increasing the effectivenes of established natural enemies of the CPB.

In our attempt to increase the effectiveness of established natural enemies, we need to study intensively the biological control potential and limitations of the natural enemies. To conduct these studies, we first need to develop rearing methods.

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[1]Research entomologist and biological technician, respectively, Yakima Agricultural Research Laboratory, Yakima, Wash. 98902; and Professor of Entomology, Department of Biology, Utah State University, Logan, Utah 84322.

[2]The year in italic, when it follows the author's name, refers to Literature Cited, p. 13.

REVIEW OF BIOLOGY

D. doryphorae is a larviparous protelean parasitic diptera (Townsend *1912*). In the field studies, the fly inserts one or, on occasions during late summer, two or more first instar larva subcutaneously into the host. Kelleher (*1960*) reported that the first instar larva remains within the haemolymph of the host and does not develop until the host descends into the soil to prepare to pupate. The tachinid has three larval instars. It completes its development in about 14 days, its larval development in 6 days, and pupates in about 8 days. The fly emerges about 6 days after the unparasitized CPB that enter the soil at the same time.

REARING THE COLORADO POTATO BEETLE

For successful rearing of *D. doryphorae* in the laboratory from September to June, we need to have the CPB host available for parasitism and potato plants to sustain the CPB.

Potato Foliage Production

Potato cultures (in different stages of development) are maintained in greenhouse benches under a 20-hr photoperiod, at night temperatures of 15° to 18°C and day temperatures of 24° to 27°C. These potatoes should be fertilized once every 2 weeks for maximum foliage production (R. E. Webb, ARS technical advisor for potato production, Beltsville, Md., personal communication). To support an initial population of 4,500 CPB larvae, we rotate the growing of potato foliage in eight greenhouse benches of 2.7 by 0.9 m, starting from seed potato cutting (1- to 2-oz size) and dense planting (15 to 20 cm apart).

Eggs

The oviposition cage is a 16-cm diameter battery jar, 20.5 cm deep, with a Saran cloth lid (fig. 1). It serves as a mating cage. Ten pairs of adult CPB and a bouquet of potato leaves are put in each cage. Since the adults eat their own eggs, especially after most of the foliage is consumed, a new bouquet is added and egg masses are removed three times a week.

The eggs are usually laid on the underside of the leaf in masses of 10 to 50 eggs. The masses are then clipped from the leaf; enough leaf material is left to permit ease of handling. The egg masses are placed into an air incubation chamber to hatch (fig. 2, cage design by T. Jermy, personal communication, Budapest, Hungary). Water at the bottom of the incubator maintains high humidity to prevent drying of the eggs. The lower portion of the chamber is covered with a nylon cloth for ventilation and to contain the emerging larvae. For faster hatching, the chambers are placed in a 24°C constant temperature cabinet with a 16-hr photoperiod for egg hatch in about 4 to 5 days. Eggs kept at room temperatures ranging from 16° to 27°C hatch in 8 or 9 days.

Figure 1.--Oviposition cages for the Colorado potato beetle with a bouquet of potato foliage.

Figure 2.--Incubator for eggs of the Colorado potato beetle with water at the bottom of the chamber.

Larvae

The larval stages of the CPB are reared in plastic containers (25 cm in diameter and 6 cm deep) (fig. 3). Unparasitized hosts are used for maintaining a continuous stock culture. The first and second larval stages are relatively easy to rear because of the small amount of leaf area (226 mm^2). The fourth larval stage consumes about 30 times more foliage than the first stage and 6 times more foliage than the second stage (Tamaki and Butt *1978*). Consequently, the larger larvae require daily maintenance of the cages. In the late fourth instar, moist sand is added to the bottom of the cages to allow the larvae to pupate. Under these rearing conditions, larvae are ready for parasitization 7 to 10 days after hatching.

REARING THE TACHINID PARASITE

Field Collection

The starter culture of *D. doryphorae* is collected from the field during the second generation when the fourth instar CPB's are most abundant, usually in late July and August in the Yakima Valley. We have encountered parasitism rates at this time ranging from 10 to 70 percent.

Puparia

Parasitized fourth instar CPB from the laboratory or field are reared in the laboratory as described. Field-collected larvae are reared in different cages. About 10 to 12 days after they enter the sand to pupate, the sand is washed away to expose puparia of the parasite and pupae of the CPB (fig. 4). The carcass of the CPB larvae can be washed away from some puparia by additional gentle spraying.

The puparia of the tachinid look like dead CPB prepupae (fig. 5). The puparia are placed in a ventilated plastic growth chamber, 72 mm in diameter and 19 mm in depth (fig. 6). About 25 puparia are placed into each chamber. Three layers of moist paper towelling are placed at the bottom of the cage for a suitably moist environment for fly emergence.

Adults

A 10-mm hole is cut near the margin of the lid of the growth chamber, to allow the emerging fly to exit. Since it walks in a circular pattern around the circumference of the cage, the fly rarely escapes although the hole is made in the center of the lid. The puparia chamber is placed in the mating cage when the adult flies are close to eclosion. The mating cage has an upper cloth

Figure 3.--Plastic cage used to rear the larval stages of the Colorado potato beetle larvae.

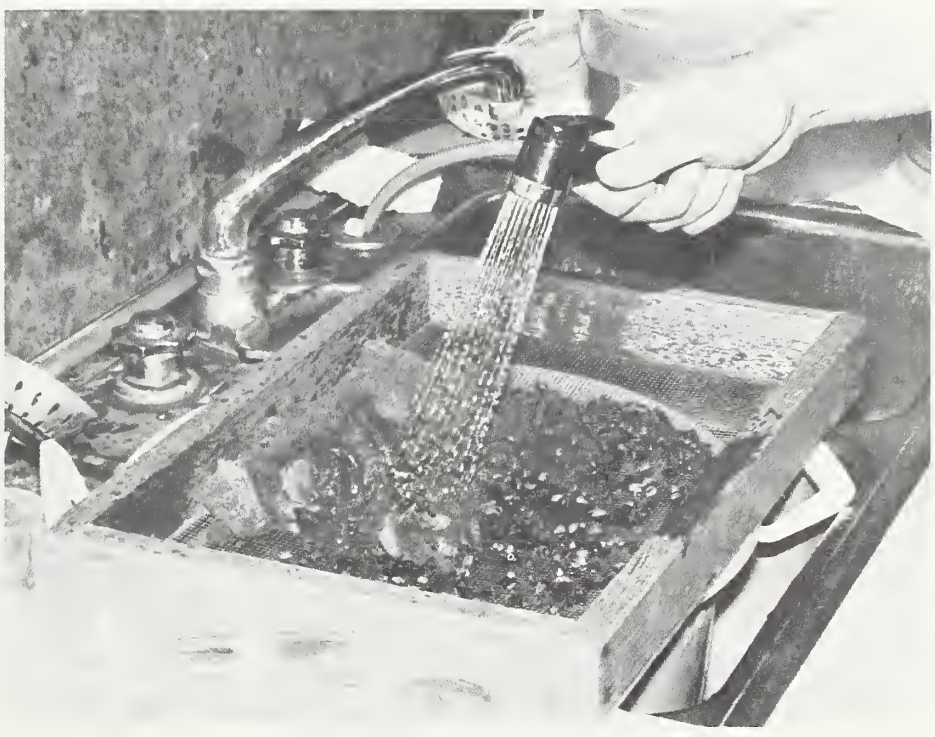

Figure 4.--Procedure to wash soil from puparia of *Doryphorophaga doryphorae* and pupae of the Colorado potato beetle.

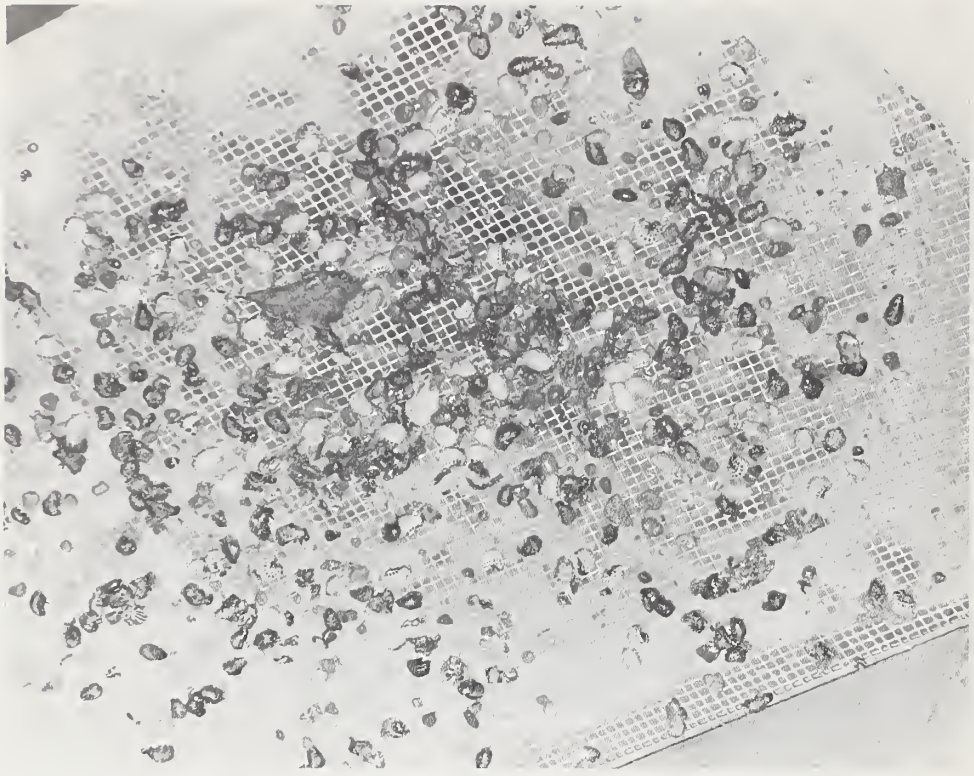

Figure 5.--Exposed puparia of tachinid fly and larvae of the Colorado potato beetle.

Figure 6.--Ventilated chambers with moist paper towelling used for holding puparia for eventual fly emergence.

section 38 cm in diameter and 40 cm high and a lower plastic bottom section lined with strips of paper towelling at the bottom to provide a suitable resting place for the flies landing near the food source (fig. 7).

The males usually emerge 2 to 3 days before the females. If sexing is necessary, the female can be readily identified by her larvipositor.

In the mating cages, the adult flies are provided moisture from a wet cotton wick and nutrition from honey smeared on paper towelling and a cube of sugar in a petri dish at the bottom of the mating cage. We used a Webb and Eckenrode (*1978*) dried diet for seed corn maggot, with 10 parts skimmed milk powder, 10 parts sugar, 1 part Brewer's yeast, 1 part soy peptone, and modified the diet with the addition of 1 part pollen. Pollen from such plants as corn and sunflower can be field collected, sifted, and placed in paper bags to be dried under a light bulb to prevent clumping and molding. The honeybee pollen purchased at health food stores should be dissolved in distilled water, the excess water taken off in a Buchner funnel under vacuum. The pollen should be further dried under a light bulb for about 48 hr and then powdered before using.

Mating

The mating cages are maintained at between 15.5° to 27°C in greenhouses with supplemented fluorescent lighting on a 16-hr photoperiod. Increase in mating frequency occurs under greenhouse conditions with access to sunlight. Attempts made to induce mating under artificial light only, with no natural light, inhibited mating. After all the flies have emerged, in 7 to 10 days, fertile females are found in the cages.

Fertility

We have observed nonfertile as well as fertile female flies attempting to parasitize the larvae of the CPB. Therefore, for an accurate determination of fertile females, each female had to be tested. The female parasite was allowed access to nonparasitized third or fourth stage instar hosts in a 150-ml snap cap vial (fig. 8). A sugar cube and a wet piece of sponge supply food and water, and an 8 by 1 cm stick provides a perch for the fly while in this vial. After 10 to 15 min of exposure to the parasite, the CPB larva is removed from the vial and dissected to search for the first instar parasite larva. The CPB larva is dissected by grasping the tip of the abdomen with forceps and decapitating the larva with a scalpel. The gut contents of the body are pushed toward the head by the blade edge of the scalpel. The entrails are examined under magnification for the parasite larva.

Once it has been determined which females are fertile, five fertile females are placed into a larger (950 ml) parasitizing cage (fig. 8). The cage has nutrients as described for the mating cage (fig. 6). The lid is at the bottom so the cage can be easily twisted and slanted to the side to introduce larvae of the CPB onto a potato leaflet, which serves to arrest the beetle larvae and is inside the cage. Individual third or fourth instar larvae are transferred by

Figure 7.--The mating cage for *Doryphorophaga doryphorae*.

Figure 8.--A small 150-ml vial used to test female fertility.

soft tweezer to the leaf in the parasitism cage and removed when stung. Initially, it takes only a few seconds for the fly to sting the larva, but as more larvae are introduced, the intervals between larviposition attempts are extended.

When large numbers of fertile female flies are available, the large mating cages shown in figure 6 can be used to parasitize a greater number of CPB larvae. About 20 larvae are placed at the bottom of the cage, and the larvae are removed and replaced every 5 min.

The procedure of removing larvae after being stung prevents superparasitism of beetle larvae, which has been observed to cause death in many instances. In our field studies, superparasitism occurs in late season when the population of CPB is low and fly density is relatively high, but in the laboratory, leaving larvae in parasitization cages can result in either death of the CPB larva or encapsulation of the parasite larvae within the CPB. In some cases, as many as 35 first instar parasites were found in a single beetle larva. Thus, rearing efficiency is greatly increased by avoiding superparasitism.

Production

The start of the fourth generation of laboratory-reared flies illustrates our rearing productivity. As shown in table 1, for example, group A CPB larvae were parasitized during a 5-day period. After 8 days, all larvae had entered the soil. Ten days later, the puparia and pupating CPB were washed and sorted. The flies emerged 13 to 21 days later. The average fly emergence or percent parasitism for all groups was 88 percent.

These emerging adults were put into mating cages held in either greenhouse or laboratory conditions. Those flies held in the greenhouse were 78 percent fertile, but no fertile flies were produced in laboratory rooms with artificial light (table 2). In earlier work, some fertile flies were produced under laboratory conditions.

Five fertile female flies were held in each cage. In successive days, from March 17 to 25, 810 larvae of the CPB were introduced into 14 parasitizing cages (fig. 9). These larvae were reared to the pupal stage then washed from the sand and sorted--parasitized from unparasitized. We retrieved 277 pupae of the CPB and 302 puparia of the tachinid parasite; 126 larvae and pupae were found dead or diseased. These rearing procedures resulted in 37 percent parasitism.

A total of 70 fertile females produced an estimated average of 4.30 puparia per day. The larviposition period of the tachinid fly is about 21.5 days (our unpublished data); therfore, each fertile female tachinid fly can produce up to 92 fly puparia in its lifetime using our laboratory procedures.

In our rearing procedure, we completed six generations starting from field collected pupae with fly emergence from about Sept. 1 to 10, 1980, and the first laboratory-reared generation completed with fly emergence from Oct. 22 to Nov. 1, 1980. Flies of the second to sixth generation emerged as follows: Dec. 1 to 15 (second), Jan. 14 to 22, 1981 (third), Feb. 20 to Mar. 10 (fourth), Apr. 20 to May 5 (fifth), and June 4 to 12 (sixth). The flies from the sixth generation were used for field release studies.

Figure 9.--Cage used to parasitize the larvae of the Colorado potato beetle.

Table 1.--*Percent fly emergence and sex ratio of the fourth generation of* Doryphorophaga doryphorae *reared under laboratory conditions*

	CPB larvae				Flies		
Group	No. parasitized	Date parasitized	Date entered soil	Date washed and sorted	Date of emergence	Female:Male sex ratio	Percent emergence
A	172	Jan. 26-30	Feb. 9	Feb. 17	Mar. 2-10	80:84	95
B	56	Feb. 4-7	14	24	1-10	28:25	95
C	176	9-13	21	Mar. 2	3-16	65:74	79

Table 2.--*Fertility rate of females of fourth generation* Doryphorophaga doryphorae

Group	No. of cages	Location of cages	Avg. No. of flies per cage		Avg. No. fertile females per cage
			Male	Female	
A	4	greenhouse	10	12	9
	4	laboratory	9	10	0
B	2	greenhouse	13	14	11
C	6	greenhouse	13	11	9

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